The Administration of **Monosodium L-Glutamate** to Neonatal & Pregnant Rhesus Monkeys 6/7/73

Q 20

GRM132

Toxicology, 1 (1973) 197 — 204

THE ADMINISTRATION OF MONOSODIUM L-GLUTAMATE TO NEONATAL AND PREGNANT RHESUS MONKEYS

A.J. NEWMAN, R. HEYWOOD, A.K. PALMER,
D.H. BARRY, F.P. EDWARDS and A.N. WORDEN

Huntingdon Research Centre, Huntingdon, PE18 6ES (Great Britain)

(Received June 7th, 1973)
(Accepted June 7th, 1973)

SUMMARY

Monosodium L-glutamate (MSG) was administered, in 3 series of experiments, to infant rhesus monkeys of both sexes in single oral doses at the rate of 2 to 4 g/kg body weight: after observation for approx. 4 h, during which there was no evidence of vomiting or other malreaction, and subsequent perfusion, examination was made by light and electron microscopy for evidence of changes in the hypothalamic region. In a fourth experiment the new-born progeny of the mothers that had received 4 g/kg body weight/day MSG during the last one-third of pregnancy were similarly examined after having been removed at birth and observed for 3 h. There was no evidence in any instance of any change that could be attributed to MSG as described by Olney and Sharpe, although there were artefacts in some inadequately fixed areas as recorded by Reynolds and her co-workers.

It is concluded that the negative effect of high oral doses, taken into account with the results of investigations by Olney and others, is consistent with the safety-in-use of MSG as a food flavour enhancer.

INTRODUCTION

MSG is employed as a food flavour enhancer, having been identified in 1908 as the main flavour component of sea tangle (Laminaria species)[1], and

Abbreviations: GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HRC, Huntingdon Research Centre; MSG, monosodium L-glutamate; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

is now produced in various countries by protein hydrolysis and by direct fermentation[2]. It has been reported that MSG administered parenterally to mice produces an acute degenerative lesion in the inner retina[3], and that neonatal mice given MSG for several months become obese[4]. Olney[5] stated that the subcutaneous injection of newborn mice with MSG induces an acute neuronal necrosis in the hypothalamus, and that adults given large doses by the same route become obese and, in the case of females, sterile. At the HRC[6] it has been found that high doses induce degenerative changes in the hypothalamus of neonatal mice or rats, by the oral as well as by the subcutaneous route, although these do not appear to be specific, but Adamo and Ratner[7] did not detect brain damage in infant rats given MSG subcutaneously.

Olney and Sharpe[8] recorded damage to the hypothalamic neurones in a neonate rhesus (*Macaca mulatta* Zimmerman), admittedly classified as premature[9], injected subcutaneously with 2.7 g MSG/kg body weight and observed for 3 h prior to sacrifice: from electron microscopy it appeared that the tissue components primarily affected were the dendrites and cell bodies of the neurones. Lowe[10] suggested that an equally plausible explanation of their findings was a non-specific solute effect, since it had been reported that the intravenous or intraperitoneal administration of many univalent completely ionized salts, given to infant cats in doses of 15 mequiv./kg body weight, reduces cerebrospinal fluid pressure to zero in 180 min, with resultant dilatation of capillaries and small vessels and occasional tearing of vessels in the Virchow—Robin space[11,12]. Zavon[13] was adversely critical of an uncontrolled single experiment. In reply, Olney and Sharpe[14] drew attention to their studies on infant mice[5,8] and emphasised that MSG had been introduced into baby foods without prior safety tests on infant animals of any species.

Reynolds and her co-workers[15] did not observe any morphological differences between the hypothalamic region of infant rhesus monkeys given MSG by stomach tube and that of control animals, but found that small areas of inadequately fixed tissue had the same appearance as that attributed by Olney and Sharpe to dosage with MSG.

Abraham and co-workers[16], while confirming lysosomal changes in the arcuate nucleus of mice given MSG, also failed to detect hypothalamic cytopathology in 4 infant rhesus monkeys given this compound.

Olney and his colleagues[17] subsequently examined the brains of 6 rhesus monkeys, 1 to 7 days old, that had been dosed with MSG, and those of 3 controls dosed with sodium chloride. They stated that those given 1 or 2 g MSG/kg body weight orally sustained small focal hypothalamic lesions confined primarily to the rostro-ventral aspect of the infundibular nucleus, that in two animals given 4 g/kg orally the lesions spread throughout and sometimes beyond this nucleus, and that one receiving 4 g/kg subcutaneously exhibited cyanosis and convulsions during the 5 h prior to sacrifice. From blood glutamate curves they concluded that the threshold for lesion formation was of the order of 20 mg/100 ml. They discarded the fixation artefact

hypothesis[15] on the grounds that the observed changes, in particular the clumping of nuclear chromatin and nuclear pyknosis, were manifestations of the necrobiosis process, *i.e.* spontaneous death of neurones during natural development.

The present series of investigations was commenced following the original publication of Olney and Sharpe[8], in order to provide controlled observations on a more adequate scale.

METHODS

Rhesus monkeys were maintained and observed in the primate buildings of HRC, where most of them were bred. MSG was supplied by the Ajinomoto Company, Tokyo, through the courtesy of Dr. Toshinao Tsunoda and his colleagues, and conformed to the specifications of the Japanese Standard of Food Additives: it was administered as a 20% w/v aqueous solution by intragastric intubation.

For the first or initial study on 4 infant monkeys, one (male, 108 days of age, body weight 1100 g) served as a negative control and the others (male, age 99 days, weight 1250 g; female, age 60 days, weight 650 g; and female, age 3 days, weight 350 g, respectively) were given single individual doses equivalent to 2 g MSG/kg body weight. Approximately 4 h after dosing, each animal was tranquilized by the intramuscular injection of 2 mg phencyclidine hydrochloride and anaesthetised by the intravenous injection of 0.2 ml phenobarbitone sodium. 500 I.U. heparin was administered intravenously, the thoracic and abdominal cavities were opened, and the animal perfused *via* the left ventricle with 10% buffered formalin for 30 min, care being taken to clamp the descending aorta. The brain was then removed and placed in 10% buffered formalin. After fixation, a block of tissue, bordered rostrally by the optic chiasma and caudally by the pons, and including the hypothalamic region, was removed and processed in paraffin wax (M.P. 56°). Serial sections, 10 μ thick, were cut at 50-μ intervals in the horizontal plane and stained with haematoxylin and eosin for examination by light microscopy.

For the second study, 16 animals were divided into 5 groups (see Table I). The test animals in the first 4 groups received a single dose of 2 g MSG/kg, and those in the fifth group 4 g MSG/kg. The subsequent procedures were as described for the initial study except that, additionally, serum and plasma samples were obtained prior to dosing and to sacrifice, and examined for SGPT and SGOT by the methods outlined in Sigma Bulletin 505 and discussed by Worden[18], and for plasma glutamic acid by electrophoretic separation followed by colorimetric estimation[19]. Also, at sacrifice, a sample of liver was removed and deep frozen. Following maceration of the liver sample, the GPT and GOT were determined by methods essentially similar to those adopted for SGPT and SGOT.

For the third study, the animals were born at HRC from 10 mothers that had been determined as being pregnant by rectal palpation on the 20th day

TABLE I
EFFECT OF MSG ON SERUM AND LIVER GPT AND GOT VALUES AND BRAIN MORPHOLOGY OF INFANT MONKEYS

Group and MSG dosage	Animal	Age (days)	Weight (g)	Pre-dose values		Terminal values							Histopathology
				Serum		Serum		Plasma[c] glutamic acid	Liver				
				GPT[b]	GOT[b]	GPT[b]	GOT[b]		GPT[d]	Glutamate[e]	GOT[d]	Glutamate[e]	
	EA[a]	5	450	13	112	17	75	0.263	6800	197	20100	581	Negative
(1)	DX	4	370	43	104	11	54	0.302	5100	148	39600	1150	Small nest glial cells adjacent to third ventricle
2 g/kg	DY	5	400	—	—	9	174	0.243	7400	214	17300	501	Negative
	DZ	4	400	—	—	8	181	0.405	5200	151	17950	519	Small increase of glial cells adjacent to third ventricle
	FG[a]	9	450	30	50	36	48	0.139	10400	301	37800	1095	Small nest glial cells adjacent to third ventricle. Occasional hyperchromatic nucleus
(2)	FP	10	400	30	62	26	26	0.363	6600	191	41800	1211	Occasional hyperchromatic nucleus
2 g/kg	FQ	9	450	30	78	23	41	0.230	6800	197	36000	1043	Negative
	DG[a]	20	450	34	240	19	—	0.158	13800	399	26300	761	Small nest of glial cells adjacent to third ventricle
(3)	DT	21	560	16	—	11	61	0.211	6900	199	21000	608	Negative
2 g/kg	FK	20	600	30	63	20	31	1.735	6400	185	43000	1245	Occasional hyperchromatic nucleus
	FB[a]	40	600	30	48	19	35	0.260	6200	179	36200	1048	Negative
(4)	DP	46	640	12	95	16	44	0.239	8500	246	20700	598	Negative
2 g/kg	EZ	44	600	33	91	32	42	0.095	13600	394	46400	1342	Occasional hyperchromatic nucleus
	DE[a]	80	700	26	95	18	102	0.289	15400	446	16100	465	Negative
(5)	DJ	80	620	15	55	10	36	0.330	8500	246	20900	598	Negative
4 g/kg	DK	80	600	26	85	12	—	0.303	11900	344	18700	540	Small nest of glial cells adjacent to third ventricle

a Control
b Sigma-Frankel units/ml
c mg glutamic acid/ml plasma
d Sigma-Frankel units/g
e μM glutamate per g per hour formed by GPT or GOT present

TABLE II
MATERNAL BODY WEIGHTS OF MONKEYS FED 4 mg MSG/kg BODY WEIGHT DURING LAST ONE-THIRD OF PREGNANCY

Group	Animal No.	Day 20 (pregnancy)	Day 100 (pregnancy)	After parturition
Undosed controls	11	4.800	4.800	4.800
	A	5.300	5.600	5.500
	19	6.000	6.500	6.100
	F	6.600	7.600	7.400
MSG	21	4.200	5.000	3.900
	C	8.100	8.550	9.000
	15	4.860	5.350	4.900
	20	4.800	5.200	4.800
	153	8.500	9.800	8.500
	5	6.800	7.250	5.800

of gestation: the maternal body weights are given in Table II. 4 of the mothers served as the source of control offspring and the other 6 each received 4 g MSG/kg body weight/day during the last one-third of pregnancy, the daily dose being administered as a solution in 500 ml water. The test solution was readily consumed voluntarily by all animals on all occasions throughout the study. All births were normal and single (Table III). After birth, each offspring was removed as soon as possible from its mother, observed for 4 h, and then treated as in the first study.

For the fourth study, 4 monkeys, each 2 days of age, were employed. 2 served as negative controls while the others were given single doses of 2 g MSG/kg body weight and, after 4 h, treated as those in the initial study

TABLE III
DURATION OF GESTATION AND BIRTH WEIGHT OF INFANTS OF UNDOSED CONTROLS AND OF MOTHERS GIVEN 4 mg MSG/kg/DAY

Group	Animal No.	Dosing period (days to end of gestation)	Duration of gestation	Infant	
				Sex	Weight (g)
Undosed controls	11	—	161	♀	406
	A	—	170	♂	407
	19	—	174	♂	507
	F	—	177	♂	563
MSG	21	45	165	♀	430
	C	50	167	♀	472
	15	51	169	♀	434
	20	51	167	♂	440
	153	56	165	♀	400
	5	64	164	♂	400

c [illegible]
d Sigma-Frankel units/g
e μM glutamate per g per hour formed by GPT or GOT pre

except that the perfusion fluid was a 2.5 v/v aqueous solution of glutaraldehyde and that the brains after removal were stored for a further 4 h in a similar solution[20]. The brains were then bisected along the line of the central sulcus and third ventricle, one-half being placed for 48 h in 10% buffered formalin for study by light microscopy and the other half fixed overnight in glutaraldehyde for electron microscopy. Excess glutaraldehyde was removed by washing in 0.2 molar phosphate buffer at pH 7.2, and secondary fixation carried out in a 1% w/v solution of osmium tetroxide in the same buffer. Cubes of tissue with sides of 2 to 3 mm were dehydrated through ascending grades of ethanol and impregnated with epoxy resin. Survey sections 1 mμ thick were stained with toluidine blue[21] to allow orientation of the tissues. Selected areas were then sectioned at 60 to 150 nm and were collected on copper grids before staining with uranyl acetate and lead hydroxide[22]. The grids were examined using a Phillips EM 300 and photographs taken at a magnification of 3000.

RESULTS

First study. All 4 animals survived the period of 3.5 to 4 h between treatment and sacrifice without obvious malreaction. Histological examination of sections of the hypothalamus failed to reveal any evidence of neuronal damage. The 3-day-old monkey had a few hypochromatic nuclei, and a minimal degree of vacuolation in the ventral hypothalamus, but these findings were not regarded as significant.

Second study. There was no evidence of malreaction during the 4-h observation period between dosing and sacrifice. The biochemical and histological findings are summarised in Table I, from which it will be seen that all results may be considered to lie within the normal ranges and that there were no changes in the hypothalamus that could be considered to be associated with the administration of MSG. In two of the animals in Group 1, predosing serum samples could not be obtained, and the high terminal SGOT levels in these monkeys are regarded as indicative of tissue damage during the attempts to obtain the samples. Plasma glutamic acid, liver GPT and liver GOT values were all within the normal range for monkeys of this age. There were not any findings in any of the sections of brain examined considered to be associated with the administration of MSG. Small nests of glial cells were seen, but as these are regularly noted in neonate monkey brains they were regarded as being without significance, as was the occasional hyperchromatic nucleus.

Third study. During the first 4 to 5 days following the initiation of treatment, loose faeces were recorded in some of the adult females, while in two cases there was food refusal for 3 days. Faecal consistency and food consumption returned to normal almost immediately, and there were no further signs of any kind. The duration of the pregnancies, all within the accepted range at HRC of 166.8 days ± S.D. 4.98, and the birth weights of the infant monkeys are recorded in Table III. Three control mothers had longer gesta-

tional periods than any member of the group given MSG, but all gave birth to males. The accepted gestation period for the production of male infants of this species at HRC is 170 days ± 4.72 S.D. There were not any cases of delayed parturition or dystocia, while nursing, suckling and other behavioural patterns were normal except for monkey 15, which killed its infant at birth.

Infant birth weights were all within the normal range at HRC of 440 g ± 50.37 S.D. Male birth weights were heavier, and again within the accepted range of 452 g ± 67 S.D. All infants behaved normally. Neither macroscopic nor histological abnormalities were detected, and serial sections of the hypothalamus appeared normal.

Fourth study. All animals survived the 4 h between treatment and sacrifice without any sign of malreaction.

Serial sections of the hypothalamus and related structures did not reveal any abnormalities when examined by light microscopy. By electron microscopy, changes of the type reported by Olney and Sharpe[8] were seen in both test and control animals, and were attributed to fixation artefact[15]. Areas of poor fixation were frequently found adjacent to areas of adequate fixation, and in them the changes included variation of chromatin pattern in the cell nuclei, and swelling of neuroglial and dendritic processes.

DISCUSSION

There was no indication of damage associated with the administration to young monkeys of single oral doses of MSG of up to 4 g/kg body weight, or with the continued administration of MSG to the dams during the last third of pregnancy. There were, however, artefacts in portions of the brain that had been inadequately fixed, consistent with the changes reported by Reynolds and her co-workers[15]. It would appear likely that in the young monkey a dosage level of 4 g MSG/kg body weight is without effect orally.

Other studies at HRC[6] have confirmed that, in neonatal rats and mice, single large doses of MSG may produce degenerative changes in the lateral ventricle and ventral to the third ventricle, involving the arcuate nucleus and median eminence[7,16], but without affecting survival rate.

The failure of high oral doses of MSG to produce lesions in the monkey lends support to the safety-in-use of this compound as a food flavour enhancer.

REFERENCES

1 K. Ikeda, *J. Tokyo Chem. Soc.*, 30 (1909) 820.
2 A. Kuninaka, M. Kibi and K. Sagaguchi, *Food Technol.*, 19 (1964) 287.
3 D.R. Lucas and J.P. Newhouse, *Am. Med. Ass. Arch. Ophthalmol.*, 58 (1957) 193.
4 A.I. Cohen, *Am. J. Anat.*, 120 (1967) 319.
5 J.W. Olney, *Science*, 164 (1969) 719.
6 A.K. Palmer, Elizabeth A. Thomas and P.H. Hague, *Huntingdon Research Centre Report 3310/70/122*, 1970.

7 Norma J. Adamo and A. Ratner, *Science*, 169 (1970) 673.
8 J.W. Olney and L.G. Sharpe, *Science*, 166 (1969) 386.
9 S.R. Napier and P.H. Napier, *A Handbook of Living Primates*, Academic Press, New York, 1967.
10 C.U. Lowe, *Science*, 167 (1970) 1016.
11 L. Finberg, C. Luttrell and H. Redd, *Pediatrics*, 23 (1959) 46.
12 L. Finberg, *Advan. Pediat.*, 16 (1969) 325.
13 M.R. Zavon, *Science*, 167 (1970) 1017.
14 J.W. Olney and L.G. Sharpe, *Science*, 167 (1970) 1017.
15 W. Ann Reynolds, M. Lemkey-Johnson, L.J. Filer and R.M. Pitkin, *Science*, 172 (1971) 1342.
16 R. Abraham, W. Dougherty, L. Golberg and F. Coulston, *Exptl. Mol. Pathol.*, 15 (1971) 43.
17 J.W. Olney, L.G. Sharpe and R.D. Feigin, *J. Neuropath. Exptl. Neurol.*, 31 (1972) 464.
18 A.N. Worden, *The Toxicity of Compounds of Veterinary and Agricultural Importance*, Thesis, University of London, 1969.
19 E.W. Yemm and E.C. Cocking, *Analyst*, 80 (1955) 209.
20 V. Karlsson and R. Schultz, *J. Ultrastruct. Res.*, 12 (1965) 60.
21 K.C. Richardson, L. Jarrett and E.H. Finke, *Stain Technol.*, 35 (1960) 313.
22 G. Millonig, *J. Biophys. Biochem. Cytol.*, 11 (1961) 736.
23 E.S. Reynolds, *J. Cell Biol.*, 17 (1963) 208.

LEMKEY-JOHNSTON, N., V. Butler* and W. A. Reynolds, Illinois State Pediatric Institute and Department of Anatomy, University of Illinois, Chicago, Illinois. Brain damage in neonatal mice following high dosages of monosodium glutamate (MSG), N_aCl and sucrose.

Oral administration of MSG at high levels (1-4mg/g) to neonatal mice results in damage to numerous brain areas, including the arcuate-preoptic area, inferior colliculus, hippocampus, anterolateral thalamus, habenula, subfornical organ and area, postrema. (Lemkey-Johnston and Reynolds, 1973).

To ascertain the effects of osmolality in eliciting this damage, we have given oral loads of N_aCl and sucrose solutions at levels equimolar to 4 mg/g of MSG to neonatal mice. Serial sections of entire brains embedded in paraffin were examined. The young mouse (5 days) given N_aCl exhibited edema and pyknotic nuclei in the habenula, caudate-putamen, hippocampus, parahippocampus and cerebral cortex, but not in the arcuate area. The lesions radiated from foci within neural structures in contrast to a band of damage appearing to diffuse inward from cerebrospinal fluid seen characteristically in brains following MSG administration. Mean plasma levels of sodium rose from control values of 130 mEg/l to 151 and 155 mEg/l for MSG and saline-treated neonates, respectively. No lesions were seen in animals older than 6 days or in those mice given sucrose.

The pattern of damage within brain structures differed depending on whether N_aCl or MSG was used, but the type of damage was the same, viz. pyknosis and edema. Thus, the role of high sodium levels plus the extent of immaturity of the neonatal mouse present additional variables to be considered in determining the etiology of the brain lesions following MSG administration.

for Anatomists

W Alan Reynolds

Brain Research, 59 (1973) 181–189

GLUTAMATE ACCUMULATION IN INFANT MOUSE HYPOTHALAMUS: INFLUENCE OF TEMPERATURE

V. J. PEREZ, J. W. OLNEY AND SANDRA J. ROBIN

Neuropsychology Laboratory, Department of Psychiatry, Washington University School of Medicine, St. Louis, Mo. 63110 (U.S.A.)

(Accepted February 16th, 1973)

SUMMARY

In 4-day-old mice monosodium glutamate (MSG) was injected subcutaneously at a dose of 2 mg/g body weight. Immediately thereafter mice were randomly returned to the nest or isolated at room temperature of 23 °C. At 0 (control) 15 and 30 min, and 1, 2, 3, 6, 9, 12 and 18 h after injection mice were decapitated, heads frozen and blood collected. In a second group of similarly treated mice, some were isolated individually either at 23 °C or 33 °C and killed 3 h after injection. Control mice received no injections. Glutamic acid (Glu) was measured in samples of arcuate nucleus (ARH), lateral nucleus of the thalamus (LT) microdissected from frozen-dried sections of brain, and in plasma in all mice. Consistent with prior findings[21], marked increases in Glu concentration occurred in ARH of MSG-treated mice but not in LT. In the ARH of mice returned to the nest, Glu reached maximal concentrations at 1 h and steadily declined to approach normal values by 3 h. However, in mice isolated at 23 °C Glu levels in the ARH continued to rise after 1 h to reach peak values at 3 h and remained substantially elevated 6 h after treatment. Pups isolated at 23 °C had considerably higher Glu levels in ARH at 3 h than those isolated at 33 °C. Plasma concentrations of Glu tended to parallel those in the ARH of mice in corresponding groups. The importance of temperature and of rigidly controlling it during the measurement of neurochemical events in the immature organism is emphasized.

INTRODUCTION

Glutamic acid (Glu) excites neurons of the mammalian central nervous system (CNS) when administered by microelectrophoresis[3,4,6,7]. When given orally or parenterally to infant animals of several species, at doses ranging from 0.5 to 4.0 mg/g body weight, this amino acid rapidly destroys neurons in the arcuate nucleus (ARH) of the

hypothalamus[1,5,9,14,15,17,18,20] and inner layers of the retina[2,13,16,22]. Microelectrophoretic introduction of excessive Glu (5 μA × 5 min or 0.25 μA × 1 h) into the cerebral cortex of the rat produces neuronal destruction at the site of introduction[23]. Other amino acids which have both acidic and neuroexcitatory properties like Glu, namely, aspartic, cysteic, cysteine sulfinic, homocysteic acids and certain of their synthetic substituted analogs, also destroy ARH neurons in the infant hypothalamus when administered subcutaneously, with DL-homocysteic acid and the N-methyl derivatives of DL-glutamic and DL-aspartic acids being 5–100 times more potent as neurotoxins than either the L- or D-isomers of Glu[19]. The typical hypothalamic lesion is produced by administering either the amino acid (Glu) or its salt, monosodium glutamate (MSG).

In recent experiments[21] we found that Glu accumulates in the ARH of the hypothalamus but not appreciably in the adjacent ventromedial nucleus (VMH) or more distally in the lateral nucleus of the thalamus (LT) in 4-day-old mice following a single subcutaneous injection of MSG, 2 mg/g body weight. Glu reached peak concentrations in ARH at 3 h following injection. In these experiments infants were removed from the maternal nest and injected, then kept in individual open containers under a small lamp until sacrificed. In a subsequent pilot experiment we observed that when infants were returned to the maternal nest in the interval between treatment and sacrifice the elevation of Glu in ARH at 3 h was much less striking. The present investigation was undertaken to further clarify possible differences in Glu accumulation in ARH of infants returned to the maternal nest *versus* those isolated following MSG injection, and to explore the effects of temperature on Glu accumulation by ARH of the infant mouse.

MATERIALS AND METHODS

Animals and treatment

Twenty-nine litters of 261 male and female Cox Swiss albino mice (Laboratory Supply Co., Indianapolis, Ind.) were used. About half of these were born in the lab while the others were purchased as litters, 3 days old on arrival in the lab. Litters were housed at 23 °C in polycarbonate mouse cages, the floors of which were covered with cedar shavings. One litter was housed per cage. When 4 days old the mice were injected subcutaneously with an aqueous solution (10% w/v) of 2 mg of MSG (Accent, Skokie, Ill.) per g of body weight using a 30-gauge hypodermic needle and a 100-μl Hamilton syringe (The Hamilton Co., Whittier, Calif.). Control mice, randomly taken from each litter, were untreated.

Nesting or isolation after treatment

Immediately after injection some of the mice were assigned by split litter to one of two groups: (a) isolated or (b) nested. Control and experimental mice in the isolated group were placed in a plastic ice cube tray, each in a separate cubicle (1.5 in. × 1.5 in. × 1.5 in.), the bottom of which was covered with cedar shavings. Control and experimental mice in the nested group were returned to the maternally attended nest imme-

diately after injection. No deaths occurred before sacrifice in either group. At 0 (control), 15 and 30 min, and 1, 2, 3, 6, 9, 12, and 18 h after injection the mice were decapitated and the heads immediately buried in powdered CO_2. Blood was collected from the neck in heparinized capillary tubes, chilled immediately, and centrifuged to obtain plasma which was frozen in tubes of 2.5 mm inner bore. Heads and plasma were stored at —120 °C until assayed. Assays were performed on bilateral samples of the ARH and LT from 4 mice randomly taken from each of the groups except the 1 and 2 h experimental groups, each of which consisted of 8 mice. Glu in plasma was measured in 4 mice at each time except 15 and 30 min, and 1 and 2 h, when plasma from each of 8 mice was analyzed.

Additional untreated controls were isolated for 1, 3, 6, 12, and 18 h and the tissues and plasma collected as already described to assess the effects of isolation for these times on endogenous levels of Glu in the ARH, LT, and plasma. Tissues and plasma from 4 mice at each of these times of isolation were used.

Temperature

To study the influence of temperature on the accumulation of Glu by the infant ARH, ice trays were put in a 37 °C water bath and weighted so that the water level reached the edges of the trays. Other identical trays were left at 23 °C room temperature. The water bath was covered with a single thickness of cheese cloth to reduce the circulation of air in the bath and to better simulate a nesting condition. The temperature on the surface of the cedar shavings in the trays in the 37 °C bath was 33 °C. Experiments were not begun until the temperature in the cubicles was stable at 33 °C. Mice were either untreated controls or injected as already described and assigned to one of two groups, namely, 23 °C-isolated or 33 °C-isolated. Each group contained 12–14 untreated controls and 12–14 MSG-injected mice. Brains from 5 mice in each control and experimental group, and plasma from 10 in each group were taken randomly for measurement of Glu. Since it was previously demonstrated[21] that Glu accumulates maximally in the ARH 3 h after injection, the control and MSG-treated mice in each of these two groups were killed 3 h after injection and the brains and blood handled as just described.

Histology

Four mice randomly taken from the nested group and 4 from the isolated one were sacrificed by perfusion fixation 3 h after MSG injection and the brains processed for histological examination as described elsewhere[17,18].

Preparation of tissues and chemical assays

Frozen-dried sections[10] of brain, 40 μm thick, were stored *in vacuo* at —30 °C until samples of ARH and LT, weighing 0.09 –0.35 μg, were microdissected from them as described elsewhere[21]. Glu was measured in the 15 and 30 min, and 1 and 3 h plasma samples as outlined by Lowry and Passonneau[12]. Enzymatic cycling of NADP[11] was used to measure Glu in the remaining plasma samples and in all brain samples with the modifications indicated previously[21].

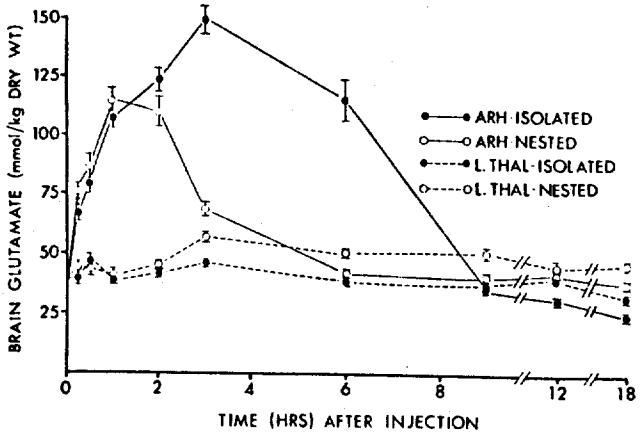

Fig. 1. Concentrations of Glu in the ARH and LT in 4-day-old mice at regular times after a single subcutaneous injection of MSG, 2 mg/g body weight. Each point represents the mean Glu (mmoles/kg dry weight ± S.E.M.) for bilateral samples of the ARH and LT from each of 4 mice, except at 1 and 2 h, where tissues from each of 8 mice were used.

RESULTS

Nested and isolated infants compared

Data are expressed as mmoles of Glu/kg dry weight of tissue (ARH and LT) and m*M* in plasma. Fig. 1 illustrates the results obtained by measuring Glu in the ARH and LT in infant mice isolated from, or returned to the nest following injection. Under both sets of experimental conditions a striking accumulation of Glu occurred in the ARH but not in the LT. The curves for Glu accumulation in the ARH of isolated and nested animals, however, had a distinctly different shape. During the first hour after injection Glu accumulated at approximately the same rate in both groups, with the 1-h values being grossly elevated at approximately 110 mmoles/kg. Thereafter, there was a steady *decline* of Glu content in the ARH of nested animals, to 68 mmoles/kg at 3 h and to 38 mmoles/kg at 6 h, in contrast to *rising* concentrations reaching a peak of 148 mmoles/kg at 3 h and remaining elevated at 114 mmoles/kg at 6 h in the isolated infants. It was of interest that Glu levels in the ARH and LT of infants isolated after MSG treatment fell somewhat between 9 and 18 h to levels less than those measured at any time in infants returned to the maternal nest after injection (35.2–24.8 mmoles/kg in the ARH, and 38.4–32.2 mmoles/kg in the LT). This probably reflects an effect of prolonged isolation and accompanying fasting since similar Glu decreases occurred during this time (not shown in Fig. 1) in the ARH (39.7–23.8 mmoles/kg) and LT (40.5–32.4 mmoles/kg) in untreated but isolated controls in which endogenous levels of Glu were measured.

Plasma concentrations of Glu in isolated and nested groups at regular times after injection are illustrated in Fig. 2. Except for the greater Glu concentration measured in plasma of nested than isolated mice at 15 min the results parallel those obtained in the ARH from both groups, *i.e.*, plasma Glu concentrations in isolated mice remained elevated for several hours after those in nested animals returned to

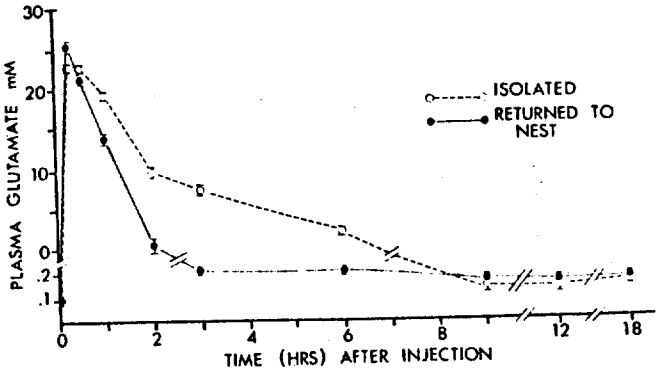

Fig. 2. Plasma concentrations of Glu in 4-day-old mice as a function of time after injection of MSG, 2 mg/g body weight. Each point represents the mean Glu (mM ± S.E.M.) in plasma of 4 mice except at 15 and 30 min, and 1 and 2 h, where Glu was measured in the plasma from each of 8 mice.

normal (0.10 mM). Unlike the gradual decrease in Glu in the ARH and LT of untreated control isolates during the 9–18 h period, plasma Glu in experimental mice remained relatively unchanged, being 0.10, 0.11, and 0.13 mM at 0, 12, and 18 h, respectively.

Temperature

Glu concentration in the ARH, LT, and plasma of experimental isolated infants subjected to regulated temperature conditions after treatment (23 °C or 33 °C) are compared in Table I with untreated control littermates under similar conditions, and

TABLE I

CONCENTRATION OF GLU IN ARH, LT AND PLASMA OF 4-DAY-OLD MICE UNDER DIFFERING ENVIRONMENTAL TEMPERATURE CONDITIONS

Glu concentrations were measured in ARH, LT and plasma of untreated controls (C) or in experimentals (E) treated subcutaneously with MSG (2 mg/g) 3 h previously. Infants were grouped to permit comparisons according to environmental temperature conditions. Group I: environmental temperature (uncontrolled) of maternally attended nest. Group II: environmental temperature controlled at 33 °C; infants isolated. Group III: environmental temperature controlled at 23 °C; infants isolated. Glu values given as mean ± S.E.M. Figures in parentheses refer to number of animals.

Groups	*Plasma (10) (mM)*		*ARH (5) (mmoles/kg dry wt.)*		*LT (5) (mmoles/kg dry wt.)*	
	C	*E*	*C*	*E*	*C*	*E*
I. Maternally nested*	0.17 ± 0.01	0.25 ± 0.04	39.7 ± 1.5	68.5 ± 2.4	40.5 ± 1.2	57.7 ± 1.62
II. Isolated, 33 °C	0.21 ± 0.01	0.22 ± 0.02	40.8 ± 1.9	67.0 ± 3.3	47.7 ± 1.9	58.9 ± 2.0
III. Isolated, 23 °C	0.16 ± 0.01	7.40 ± 0.91	34.0 ± 2.4	156.4 ± 9.0	34.0 ± 2.3	47.0 ± 0.9

* Maternally nested (E) are 3 h values from curves in Figs. 1 and 2. N = 5 (plasma) or 4 (ARH and LT).

with additional littermates experiencing the relatively warm but uncontrolled temperature of the maternal nest. Only the MSG-treated infants experiencing both isolation and a relatively low temperature (23 °C) had grossly elevated Glu concentrations in the ARH at 3 h. Experimental mice which were isolated but kept warm (33 °C) had only moderately elevated Glu concentrations in the ARH (67 mmoles/kg) at 3 h as did those returned to the maternal nest (68 mmoles/kg). Thus, the striking tendency of MSG-injected infants isolated at room temperature (23 °C) to develop *sustained* high Glu concentrations in the ARH was not seen in other MSG-injected infants, either those returned to the maternal nest or those left isolated at a regulated temperature of 33 °C.

The tendency for plasma Glu to remain elevated at 3 h in MSG-treated infants isolated at 23 °C room temperature also was absent both in infants returned to the maternal nest and those isolated at 33 °C. It should also be noted that untreated control mice, regardless of environmental circumstances, tended to have higher resting levels of Glu in the LT than ARH and that, when maintained at 33 °C the controls tended consistently to have greater Glu concentrations in the ARH, LT and plasma than when maintained at room temperature (23 °C).

Histology

Examination of stained sections, illustrated in Fig. 3a and b, of brain from animals sacrificed by perfusion fixation 3 h after MSG injection revealed similar appearing ARH lesions in both the isolated and maternally nested groups.

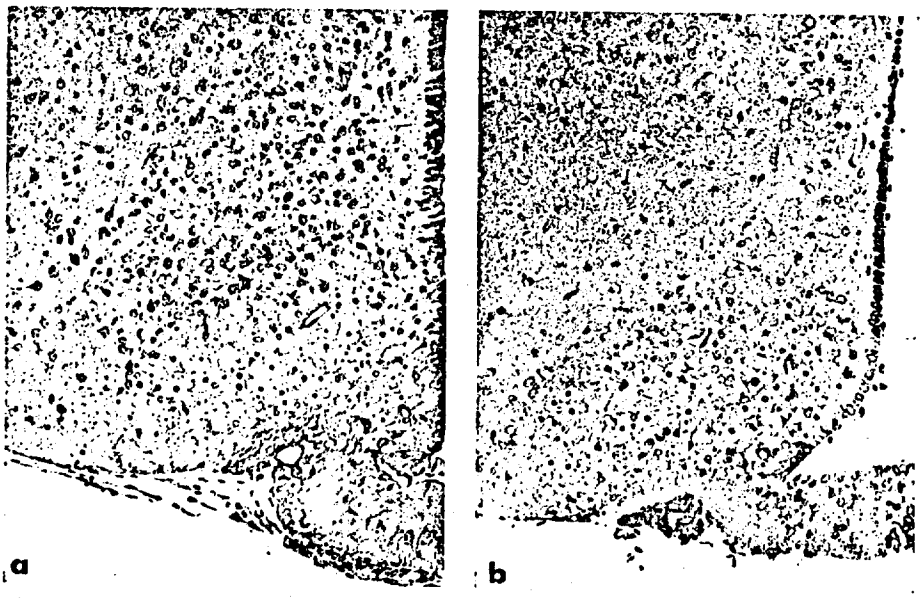

Fig. 3. a and b: coronal sections of brain from 4-day-old mice taken 3 h after subcutaneous injection of MSG, 2 mg/g body weight. a, from an infant returned to the maternal nest following injection, reveals a lesion in the ARH of the hypothalamus which does not differ appreciably from the lesion in the ARH of an infant isolated after injection (b). Brains were fixed, sectioned and stained according to histological procedures described by Olney[18]. Magnification × 100.

DISCUSSION

Evidence presented here confirms that leaving infant mice isolated at room temperature following an injection of MSG is conducive to the development of higher and more sustained Glu elevations in the ARH than occur in infants returned to the maternal nest following injection. Apparently, the difference in Glu accumulation curves can be explained almost entirely in terms of a body temperature differential since Glu concentrations in the ARH 3 h after injection were not different for nested and isolated infants provided the latter were maintained at 33 °C rather than at 23 °C. It is reasonable to assume that body temperature, whether maintained by contact of the infant with its mother or by artificial means, might influence the rate of metabolic reactions involving Glu, not only centrally within the ARH but peripherally, *e.g.*, in the liver and blood. A slower rate of metabolism peripherally may underly the more sustained elevation of plasma Glu in infants isolated at room temperature and this, in turn, could account at least partially for the higher and more prolonged ARH elevations of Glu in these infants. That is, increasing the time during which plasma Glu remains elevated above some critical threshold level would extend the time during which uptake of the amino acid by the infant ARH would be possible. Probably both a prolonged uptake of Glu by the ARH and a reduced rate of metabolic processing of Glu within the ARH contributed to the higher and more sustained elevations of Glu observed in the ARH of infants isolated at 23 °C. Further studies will be needed to establish the role played by the activities of specific enzyme systems in producing this effect.

In a previous study[21] we presented a curve for Glu accumulation in the ARH following MSG injection similar in shape to that shown here (Fig. 1) for infants isolated at room temperature (23 °C). In both experiments infants were isolated in a room maintained at 23 °C and Glu concentrations peaked in ARH at 3 h. However, the 3 h value reported previously (112 mmoles/kg) was not as high as is reported now (156 mmoles/kg), probably because the infants' surroundings were kept somewhat warmer than 23 °C by a small lamp in the prior study. In other experiments (Perez *et al.*, unpublished), infant mice were isolated from their mother following MSG treatment in a room at 23 °C but were grouped in a small container so that constant contact with one another was maintained. Glu levels in ARH at 3 h were lower (116 mmoles/kg) for these grouped infants than we report here for infants isolated individually at room temperature (156 mmoles/kg) but higher than in infants returned to the maternally attended nest (68 mmoles/kg). The intermediate value for infants grouped together probably is indicative of their ability to conserve body heat more effectively than their counterparts isolated individually, but less effectively than those attended by their mother. We also grouped infants in the absence of the mother but at a temperature of 33 °C and the Glu concentration in the ARH 3 h after treatment was 60 mmoles/kg, *i.e.*, about the same as when the pups were warmed by their mothers (68 mmoles/kg). These observations and others from the present study all support the thesis that Glu accumulation in the ARH of infant mice becomes a more prolonged

process involving more extreme Glu elevations as ambient body temperature shifts from warm to relative coolness.

It is of interest, despite the marked difference in pattern of Glu accumulation in the ARH of nested and isolated groups of infants that the ARH lesions observed in stained histologic sections from these two groups (Fig. 3a, b) did not appear different. Tentatively, we suggest that this may be a result of the relatively high dose of MSG used (2 mg/g), *i.e.*, it may have been great enough to produce peak concentrations of Glu in ARH substantially exceeding the toxic threshold of ARH neurons under either set of conditions. Glu concentrations in ARH apparently do not have to remain elevated very long for necrosis of ARH neurons to occur since evidence of neuronal degeneration is already detectable 30 min after a 2 mg/g injection of MSG[18]. To establish that low body temperature renders an infant animal more vulnerable to brain damage from MSG, or conversely, that higher body temperatures may be protective will require additional experiments designed more specifically to examine this problem.

It is well recognized that biochemical reactions in the body are affected by changes in body temperature and that the immature mammal regulates body temperature poorly. Thus, exposure of infant animals to substantially different ambient temperature conditions might be expected to influence the biochemistry of various tissues, including brain. However, except for data recently reported by Lajtha[8] very little has been studied in this respect. Lajtha's group found that incorporation of radioactive valine by infant mouse brain *in vivo* occurs at a more rapid rate when the temperature of the isolated infant's surroundings is maintained in the range of body temperature (37 °C) than when maintained at room temperature (25 °C). It is difficult to make direct comparisons between these data and ours in that different amino acids were studied and different experimental methods were employed. However, it is a finding common to both studies that changes in ambient temperature decidedly influence amino acid chemistry of the immature mouse brain and, it seems not unlikely that other species and other parameters of brain chemistry might be subject to ambient temperature influences as well. Although being left in the maternal nest is the most natural set of conditions for the infant mouse, it must be recognized that under the nesting situation the attentiveness of the mother remains an uncontrolled determinant of the infant's body temperature. Variations in maternal care, particularly when the mother is upset by the experimenter disturbing her nest, are the rule rather than the exception. Thus for certain types of developmental neurochemical studies the maternal nest may not provide adequate temperature controls. In any event, the marked temperature-dependent differences we show in Glu accumulation by ARH of isolated *versus* nested infant mice suggests that temperature plays an important role in regulating neurochemical events in the immature mammal and that the mother's role as thermoregulator for the infant must not be underestimated in developmental neurochemical research.

ACKNOWLEDGEMENTS

This research was supported by Grants NS-09156 and NS-08909.

J. W. Olney is the recipient of Research Career Development Award MH-38894. We thank Dr. Eli Robins for his helpful comments during the preparation of this manuscript.

REFERENCES

1 BURDE, R. M., SCHAINKER, B., AND KAYES, J., Monosodium glutamate: acute effect of oral and subcutaneous administration on the arcuate nucleus of the hypothalamus in mice and rats, *Nature (Lond.)*, 233 (1971) 58–60.

2 COHEN, A. I., An electron microscopic study of the modification by monosodium glutamate of the retinas of normal and 'rodless' mice, *Amer. J. Anat.*, 120 (1967) 319–335.

3 CURTIS, D. R., PHILLIS, J. W., AND WATKINS, J. C., The chemical excitation of spinal neurones by acidic amino acids, *J. Physiol. (Lond.)*, 150 (1969) 656–682.

4 CURTIS, D. R., AND CRAWFORD, J. M., Central synaptic transmission-microelectrophoretic studies, *Ann. Rev. Pharmacol.*, 9 (1969) 209–240.

5 EVERLY, J. L., Light microscopic examination of MSG-induced lesions in brain of fetal and neonatal rats, *Anat. Rec.*, 169 (1971) 312.

6 HAYASHI, T., Effects of sodium glutamate on the nervous system, *Keio J. Med.*, 3 (1954) 183–192.

7 KRNJEVIĆ, K., AND PHILLIS, J. W., Iontophoretic studies of neurones in the mammalian cerebral cortex, *J. Physiol. (Lond.)*, 165 (1963) 274–304.

8 LAJTHA, A., Alteration of protein metabolism and development, *Paper presented at Symposium on Drugs and Developing Brain, University of Colorado, Denver, Colorado, Dec. 18–20*, (1972).

9 LEMKEY-JOHNSTON, N., AND REYNOLDS, W. A., Incidence and extent of brain lesions in mice following ingestion of MSG, *Anat. Rec.*, 172 (1972) 354.

10 LOWRY, O. H., The quantitative histochemistry of the brain: histological sampling, *J. Histochem. Cytochem.*, 1 (1953) 420–428.

11 LOWRY, O. H., AND PASSONNEAU, J. V., Measurement of pyridine nucleotides by enzymatic cycling. In S. P. COLOWICK AND N. O. KAPLAN (Eds.), *Methods in Enzymology, Vol. VI*, Academic Press, New York, 1963, pp. 792–800.

12 LOWRY, O. H., AND PASSONNEAU, J. V., *A Flexible System of Enzymatic Analysis*, Academic Press, New York, 1972, pp. 184–185.

13 LUCAS, D. R., AND NEWHOUSE, J. P., The toxic effect of sodium L-glutamate on the inner layers of the retina, *Arch. Ophthal.*, 58 (1957) 193–201.

14 MURAKAMI, U., AND INOUYE, M., Brain lesions in the mouse fetus caused by maternal administration of monosodium glutamate, *Congen. Anom.*, 11 (1971) 171–177.

15 OLNEY, J. W., Brain lesions obesity and other disturbances in mice treated with monosodium glutamate, *Science*, 164 (1969) 719–721.

16 OLNEY, J. W., Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion, *J. Neuropath. exp. Neurol.*, 28 (1969) 455–474.

17 OLNEY, J. W., AND HO, O. L., Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine, *Nature (Lond.)*, 227 (1970) 609–610.

18 OLNEY, J. W., Glutamate-induced neuronal necrosis in the infant mouse hypothalamus: an electron microscopic study, *J. Neuropath. exp. Neurol.*, 30 (1971) 75–90.

19 OLNEY, J. W., HO, O. L., AND RHEE, V., Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system, *Exp. Brain Res.*, 14 (1971) 61–76.

20 OLNEY, J. W., SHARPE, L. G., AND FEIGIN, R. D., Glutamate-induced brain damage in infant primates, *J. Neuropath. exp. Neurol.*, 31 (1972) 464–488.

21 PEREZ, V. J., AND OLNEY, J. W., Accumulation of glutamic acid in the arcuate nucleus of the hypothalamus of infant mouse following subcutaneous administration of monosodium glutamate, *J. Neurochem.*, 19 (1972) 1777–1782.

22 POTTS, A. M., MODRELL, K. W., AND KINGSBURY, C., Permanent fractionation of the ERG by sodium glutamate, *Amer. J. Ophthal.*, 50 (1960) 900–907.

23 VAN HARREVELD, A., AND FIFKOVA, E., Light and electron microscopic changes in central nervous tissue after electrophoretic injection of glutamate, *Exp. molec. Pathol.*, 15 (1971) 61–81.